Seed Transmission of Bean Yellow Mosaic Virus in Broad Bean (*Vicia faba*)

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Key words: bean yellow mosaic virus, broad bean, seed transmission, ELISA, primary inoculum.

Broad bean has been an important legume on Shikoku-island, in Japan, especially, in Kagawa and Ehime prefectures since the 1900's. The occurrence of mosaic-diseases caused by viruses which has been reported to affect broad bean plants has become a major problem not only on Shikoku-island but in many other areas of Japan^{5,6,8)}. A field survey carried out in 1990 and 1991 on Shikoku-island, revealed that almost every broad bean plant showed mosaic and/or necrosis symptoms at the time of harvest, and that a high percentage of broad bean plants were infected with bean yellow mosaic virus (BYMV)⁷⁾. BYMV can be seed-transmitted in a small percentage of pea, broad bean, white sweet clover, and in a higher percentage of yellow and white lupin¹⁾. Kaiser⁴⁾ and Tanaka *et al.*^{8,9)} reported that the high incidence of mosaic-disease in broad bean fields could be ascribed to the fact that seeds infected with BYMV were brought in the fields and that the seeds represented a primary inoculum source in the fields. The purpose of this investigation was to analyse various factors influencing to seed-transmission of BYMV and to discuss the importance of infected seeds as one of the major sources of primary inoculum in the fields of Shikoku-island.

Two isolates of BYMV, BYMV 90-2 and BYMV 90-3, were used in this study. BYMV 90-2 was isolated from a naturally infected broad bean plant showing mosaic symptoms and BYMV 90-3 was obtained from a seedling which was infected with BYMV through seed. Both isolates of BYMV were identified as BYMV-B³⁾ from their host ranges and serological properties. BYMV 90-2 was purified according to the method of Uyeda *et al.*¹⁰⁾ The antiserum to BYMV 90-2 was prepared using a New Zealand white rabbit by injecting 13.4 mg of the purified virus in total. The titer of the antiserum was 1 : 128 in the ring test. Enzyme-linked immunosorbent assay (ELISA) was done as those described by Clark and Adams²⁾, except that polyvinyl-pyrrolidone (PVP) and bovine serum albumin (BSA) were not added to PBS-Tween.

Broad bean plants belonging to twelve varieties were inoculated with BYMV 90-2 or BYMV 90-3 in the greenhouse at the 3rd- or 4th-node stage. After the appearance of mosaic symptoms, the plants were transplanted in a field, and then the plants were covered with cheese cloth and sprayed at 10 to 15 day intervals with an insecticide. When the pods became mature and dry on the plants, the mature seeds were harvested. The fully mature seeds were sown and grown in the greenhouse until the 3rd- or 4th-node stage to observe the appearance of symptoms. Mosaic symptoms were present on the primary leaves and they became more pronounced on the compound leaves which developed later. BYMV was detected by ELISA in all the plants showing mosaic symptoms while BYMV was not detected in the plants without mosaic symptoms. The percentage of seed transmission of two BYMV isolates in twelve varieties of broad bean was calculated (Table 1). Among twelve broad bean varieties tested, BYMV 90-2 was found to be seed-borne in ten varieties while BYMV 90-3 was found to be seed-borne in five varieties. The percentage of seed transmission of BYMV 90-2 and 90-3 ranged from 0.0 to 9.2% and 0.0 to 3.7%, respectively. Both isolates could be transmitted with comparative ease through seeds of some varieties, such as Nintoku-issun, Shimizu-wase and Ryouisai-issun. On the other hand, both isolates could

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Table 1. Transmission of two isolates of bean yellow mosaic virus (BYMV) through seeds of various broad bean varieties

Variety	BYMV 90-2		BYMV 90-3	
	No. of seeds		No. of seeds	
	Tested	BYMV-detected ^{a)}	Tested	BYMV-detected
Boushu-wase	284	2(0.7) ^{b)}	214	0(0.0)
Ooshimazairai	231	18(7.8)	212	0(0.0)
Sanuki-nagasaya	187	10(5.3)	148	0(0.0)
Konpira-nagasaya	120	4(3.3)	223	3(1.3)
Longgreen	248	0(0.0)	127	3(2.4)
Uchikoshi-issun	131	1(0.8)	134	0(0.0)
Otafuku	229	0(0.0)	194	0(0.0)
Matushima-issun	70	1(1.4)	114	0(0.0)
Bashounari-issun	101	6(5.9)	74	0(0.0)
Nintoku-issun	99	2(2.0)	103	2(1.9)
Shimizu-wase	76	7(9.2)	112	3(2.7)
Ryousai-issun	110	3(2.7)	82	3(3.7)

a) The virus was detected by ELISA.

b) The percentage of seed transmission is indicated in parentheses.

Table 2. Detection of bean yellow mosaic virus (BYMV) from commercial broad bean seeds

Variety	Source ^{a)}	No. of seeds		Detection rate (%)
		Tested	BYMV-detected ^{b)}	
Sanuki-nagasaya	Kagawa('90)	219	0	0.0
Sanuki-nagasaya	Kagawa('91)	340	0	0.0
Sanuki-nagasaya	Kagawa('92)	126	3	2.4
Konpira-nagasaya	Kagawa('90)	267	6	2.2
Longgreen	—('90)	147	0	0.0
Uchikoshi-issun	Osaka('89)	193	0	0.0
Uchikoshi-issun	Osaka('90)	151	0	0.0
Otafuku	Kagawa('90)	109	0	0.0
Matushima-issun	Kagawa('90)	113	0	0.0
Matushima-issun	Kagawa('91)	40	2	5.0
Bashounari-issun	Kagawa('90)	124	4	3.2
Bashounari-issun	Kagawa('91)	80	3	3.8
Nintoku-issun	Kagawa('90)	135	1	0.7
Shimizu-wase	Ehime('90)	219	34	15.5
Shimizu-wase	Kagawa('90)	108	2	1.9
Shimizu-wase	Ehime('90)	199	10	5.0
Ryousai-issun	—('90)	74	5	6.8
Ryousai-issun	Osaka('90)	308	0	0.0

a) Year of production is indicated in parentheses.

b) The virus was detected by ELISA.

not be readily transmitted through seeds of other varieties, such as Boushu-wase, Uchikoshi-issun Otafuku and Matushima-issun.

Eighteen different samples of commercial seeds in ten broad bean varieties produced from 1989 to 1992 were checked for virus infection. The filamentous viruses detected in all the plants showing mosaic symptoms were identified as BYMV by ELISA. No virus-like particles were found in the plants without mosaic symptoms by electron microscopy. Table 2 shows that BYMV was detected in ten out of 18 samples of commercial broad bean seeds. The detection rates of BYMV were lower than 5.0% in most of the cases, whereas the detection rate exceeded 15.0% in case of Shimizu-wase (Ehime, '90).

In total 1,531 broad bean plants from ten different fields in Kagawa prefecture were tested for virus

Table 3. Occurrence of bean yellow mosaic virus (BYMV) in broad bean fields in Kagawa prefecture in early November 1991 and 1992

Date	Field examined	Variety	No. of plants		Detection rate(%)
			Examined	BYMV-detected ^{a)}	
Nov. 1991	Kitanishiyama Zentsuji	Otafuku	125	1	0.8
Nov. 1991	Kitoku Zentsuji (1)	Otafuku	105	2	1.9
Nov. 1991	Kitoku Zentsuji (2)	Sanuki-nagasaya	68	1	1.5
Nov. 1991	Senyucho Zentsuji (1)	Bashounari-issun	80	3	3.8
Nov. 1991	Senyucho Zentsuji (2)	Matushima-issun	40	2	5.0
Nov. 1991	Okada Ayauta	Otafuku	193	4	2.1
Nov. 1992	Kitanishiyama Zentsuji (1)	Sanuki-nagasaya	139	4	2.9
Nov. 1992	Kitanishiyama Zentsuji (2)	Sanuki-nagasaya	147	10	6.8
Nov. 1992	Kitoku Zentsuji	Wase	338	6	1.8
Nov. 1992	Okada Ayauta	Issun	296	11	3.7

a) The virus was detected by ELISA.

infection in early November 1991 and 1992 (Table 3). The broad bean seeds cultivated by farmers in Zentsuji, Kagawa prefecture, were sown in the nursery in October and the plants were grown in the nursery until the 2nd- or 3rd-node stage. Thereafter the plants were transplanted to the field. The filamentous viruses found in all the plants showing mosaic symptoms were identified as BYMV by ELISA. BYMV was detected in all of the 10 fields and forty-four out of 1,531 broad bean plants were infected with BYMV. No other viruses were detected in the fields. The percentage of the plants infected with BYMV in the fields tested ranged from 0.8% to 6.8%. Infection of the plants with BYMV was considered to be due to transmission through seeds because all the broad bean plants were grown in the nursery to prevent attack of aphids and were surveyed within 1 week after transplanting to the fields.

Kaiser⁴⁾ reported that seed transmission was an important mechanism for the survival and spread of BYMV in broad beans. In the studies described here it was observed that the percentage of seed transmission of BYMV ranged from 0.0 to 9.2% depending on the varieties of broad bean and isolates of BYMV used. A survey of seed transmission in commercial broad bean seeds and an early field survey of broad bean plants prior to aphid transmission showed that approximately 2.5% of the broad bean seeds were infected with BYMV. These infected plants were randomly scattered in the field (data not shown). BYMV was subsequently transmitted by aphids from infected plants to healthy plants in the field, then subsequently a high percentage of broad bean plants became infected with BYMV at the time of harvest⁷⁾. It is therefore likely that the seeds infected with BYMV represented one of the major sources of primary BYMV inoculum in the field.

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和 文 摘 要

笹谷孝英・岩崎真人・山本孝狹：インゲン黄斑モザイクウイルス(BYMV)のソラマメにおける種子伝染

ソラマメ 12 品種と BYMV の 2 分離株を用い種子伝染率を検討した。その結果、BYMV の種子伝染率は 0.0 から 9.2%で、品種および分離株の違いで変化した。次に、市販ソラマメ種子 18 サンプル(10 品種)についてウイルス汚染を調査したところ BYMV のみが検出され、種子伝染率はほとんどが 5.0%以下であったが、中には 15.0%以上と高率にウイルス汚染の確認されたサンプルもあった。また、農家自家採取種子を播種したソラマメ 10 圃場について、11 月上旬ウイルス病の発生状況を調査したところ、調査圃場すべてで BYMV のみの発生が確認され、発生率は 0.8 から 6.8%であった。よって、種子伝染により BYMV 感染したソラマメ種子が圃場内に持ち込まれ、BYMV 蔓延の 1 次伝染源となる可能性が示唆された。

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